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Molecular Modelling of Hydrogen Bonding in Urea and Dimethyl Urea

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The intermolecular bonding between molecules containing urea moieties is of interest due to the possible bidentate nature of the hydrogen bond. Experimentally, the nature of the hydrogen bond can be determined from the infra-red spectrum of the material. The C=O stretching frequency shows substantial shifts due to the additional constraints of the hydrogen bonding. To ascertain the capability of quantum mechanical methods to understand better the nature of hydrogen bonds between these types of molecules, calculations on urea, dimethyl urea and their dimers have been undertaken as well as calculations of the solid state crystal structures. The calculations involve the determination of the optimum geometries and the relative energetics of the hydrogen bond formation. Calculations of the vibrational frequencies within the quasi-harmonic approximation have also been performed and some comparison can be made with experiment. In addition to a further understanding of the fundamental nature of bidentate hydrogen bond structures, a study of the cost effectiveness of various computational methodologies has been undertaken. Calculations at the Hartree-Fock, Density Functional and MP2 levels of theory are reported and a comparison of various basis sets and treatments of electron correlation is made. The calculations have provided considerable insight into the nature of these type hydrogen bonding systems. Much of the information can be used to provide more effective parameterisation of force-fields to make possible the treatment of crystal structure and crystal growth. A density functional method using the edf1 functional appears to be one of the most cost effective methods. Despite using a small basis set it provides excellent prediction of vibrational frequencies.

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Structural insight into protein stability - bovine pancreatic ribonuclease A variants studies

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A large and growing number of diseases are recognized as conformational diseases. They share the same pathological feature, the ability to form insoluble aggregates of misfolded proteins [1]. Recent data suggest that such a conversion may be a common feature of many soluble proteins. Despite numerous studies, the mechanism of protein folding remains not well understood. Detailed knowledge of this process is crucial for preparation of protein based therapeutics and treatment of pathologies related to mutated, unstable proteins. Detailed analysis of equilibrium unfolding experiments using pressure and temperature as denaturing agent, revealed relationship between the substitution introduced in a particular position within the hydrophobic core of bovine pancreatic ribonuclease A and its thermodynamic consequences [2,3]. The goal of presented research is to uncover the structural origin of the decreased stability of RNase A single variants, that have been shown more critical for overall stability.

For structural analysis we selected five RNase A mutants: V47A, V54A, V57A, I81A and V108A. The X-ray data has been collected for crystals of mutated and wt protein at temperature 100 K on a Nonius KappaCCD diffractometer with sealed Mo tube (55kV, 30mA) and Miracol focusing collimator. The data sets have been obtained with resolution limit up to 1.4-1.8 Å and completeness better than 95%. The structures were refined using standard protocols of CNS program. The final R factors were in the range of 16-18 %.

There are no significant differences between the overall structure of wtRNase A and mutated protein. Only minor rearrangements are observed in the vicinity of the mutation position in all solved structures. However, analysis of the crystal structure of V108A molecule revealed three subdomains that are significantly shifted in comparison to the structure of wt protein. Similar shifts but much smaller were also observed for other mutants. It is probable that these subdomains correspond to the chain-folding initiation sites.

All Ile/Val→ Ala mutations have introduced changes in size of cavity found in wtRNase A. The crystallographic analysis did not revealed any water molecules inside the formed cavities. The size of the cavity seems to be the most important factor influencing stability of the mutated molecule. Analysis of other structural factors contributing to the protein stability will be discussed.

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